



Effect of *in-ovo* feeding maltose on the embryo growth and intestine development of broiler chicken

C L JIA¹, Z H WEI², M YU³, X Q WANG⁴ and F YU⁵

Northwest Agriculture and Forest University, Shaanxi 712 100 China

Received: 18 October 2010; Accepted: 9 February 2011

ABSTRACT

The present experiment was conducted to study the effects of *in ovo* feeding maltose on the embryo growth and intestine development of broiler. On the 17.5d of incubation, altogether 635 eggs were divided randomly into 5 treatments. The eggs of treatment 2, 3, 4 and 5 were injected with 1 ml 4.5 g/L NaCl solutions containing 100 g/L, 200 g/L, 300 g/L, 400 g/L maltose into the amnion fluid, while the eggs of treatment 1 as the control were only injected with 1 ml 4.5 g/L NaCl solution. The results showed that hatchling weights of *in ovo* feeding 100~300 g/L maltose solution group increased by 2.9 to 6.4% over controls. But higher maltose concentration reduced hatchability, and even resulted in all death (400 g/L maltose). The morphological examination of jejunum villus indicated on the day of hatching villus height, width and surface area of all IO feeding treatments exhibited increase compared with the control group and, in treatment 3, these were significantly increased by 17, 35 and 57%, respectively. So *in ovo* feeding found 200 g/L maltose solutions to the chicken embryo can promote the development of the jejunum villus, benefit due to enhancement in the absorption of nutrients and thus increase the weight of hatching.

Key words: Broiler, Embryonic growth, *In ovo* feeding, Jejunum, Maltose

The growth and development of embryo and chicken is depended on the digestion and assimilation of nutrients which is related to the morphological and functional development of the gastrointestinal tract. The development of the gut occurs throughout incubation (Romanoff 1960), and is anatomically complete by 16 day of incubation (Moran 1985), and after that the functional abilities of the gut begins to develop (Ferket *et al.* 2006). And just during 2–3 day after hatch, the chicken undergoes rapid development of the gastrointestinal tract (Bhattacharyya *et al.* 2007). The sooner the gut achieves its functional capacity the earlier the young chick can use dietary nutrients and attains maximum growth and development. During incubation many nutrient supplements administrated to embryo could directly or indirectly stimulate development of gut, growth of embryo and chick or duck, and improve livability (Uni and Ferket 2004, Tako *et al.* 2004, Uni *et al.* 2005). Carbohydrates were chosen as an important component because they are critical during the final stage of chick embryo development prior to pipping, and development of gut also needs higher energy (Park 1993), but very little carbohydrate remains in the egg

(Christensen *et al.* 1993). Exogenous carbohydrates may reduce this constraint.

Maltose, a hydrolysate of starch can be digested to glucose by several enzymes and absorbed. According to Moran (1985), maltase and isomaltase activities appear in the embryo on day 18 of incubation and reach their maximum 4 days after hatching. Maltose may provide substrates and co-factors that stimulate carbohydrate metabolism to supply the energy. Petersen *et al.* (2002) also demonstrated that higher levels of the substrate in the intestine can elevate activity of disaccharidases. So in this study, we selected different concentration maltose solutions to the chicken embryos using *in ovo* feeding (IOF) at day 17.5 of incubation to study the effect on development of the small intestine and the growth of embryos.

MATERIALS AND METHODS

Incubation and in-ovo feeding: Broiler eggs (800) with average egg weight of 56 ± 1.3 g were obtained from the poultry farm in the university. Eggs were incubated at $37.5 \pm 0.5^\circ\text{C}$ and 60% relative humidity and turn over 2 h. On day 17.5 of incubation, eggs were candled, and infertile or those with dead embryos were removed. Eggs (635) containing viable embryos were randomly divided into 5 groups. Egg from each group was injected with treatment

Present address: ^{1,2,3,4,5} College of Animal Science and Technology (¹e mail: lingb02177@163.com, ²e mail: weizehui7848@hotmail.com, ³e mail: yumeng492509829@163.com, ^{4,5}e mails: ffeng66@163.com).

solutions (1 ml/egg) into the amniotic fluid, which was identified by candling. The 5 groups included the following: the control group solution was 4.5 g NaCl/L (group 1), and the other treatment solutions were respectively 100 g (group 2), 200 g (group 3), 300 g (group 4), 400 g (group 5) of maltose/L (purity > 99.5%) in 4.5 g of NaCl/L. After the eggs were injected, the injection holes were sealed with paraffin wax and then replaced to incubate.

Tissue sampling: Five embryos or hatchlings from each treatments and control group were decapitated for analysis at 19, 20 day of incubation (19E, 20E) and on the day of hatch. The weights of embryo or hatchling were recorded before slaughter. The jejunum was removed and 2 cm long segments were taken and fixed in 4% neutral-buffered formalin solution for histology. In addition, on the day of hatch the number of hatchling was recorded to be calculated the hatchability.

Morphological examination: The fixed jejunum samples were dehydrated, cleared and embedded in paraffin. Serial sections were cut at 5 µm and placed on glass slides. Sections were deparaffinized in xylene, rehydrated in a graded alcohol series, stained with hematoxylin and eosin, and examined by light microscopy. Morphological measurements of villus height and width were performed with 1000 light microscope with 400D digital camera taking images. Villus surface area was calculated from villus height and width half height (Uni *et al.* 1998)

Statistical analysis: Differences between weights of embryos or hatchlings were examined by ANOVA using GLM of SAS-8.0 software, and initial egg mass were used as a covariate. The significance of differences between means was determined with the TDIFF option of the LSMEANS statement. Differences between means were considered significant at the $P < 0.05$ level. Hatchability data were analyzed by chi-squared test. The results were presented as least squares means with standard errors.

RESULTS AND DISCUSSION

Hatchability and body weight: The hatchability of treatments 2, 3 and control group were not significantly different (Table 1) but the hatchability of treatment 4 was significantly lower than control group. In treatment 5 all embryos were dead before hatch. At 19d of incubation, the embryo mass of groups 3 and 5 was significantly higher than that of group 1 and 4 (Table 1). On day 20, the embryo mass of group 2, 3 and 5 was significantly higher than that of group 1. On the day of hatch, the body weight of group 2, 3 was significantly higher than that of group 1 and 4.

Jejunal villus development: At 19 d of incubation, IOF treatments except for 5 group exhibited greater villus length and surface area compared with controls (Table 2, Fig. 1A). Villi length, width and villus surface area of the group 3 were greater than those of the control at 19 and 20 day (Table 2, Fig. 1B). On the day of hatch IOF treatments except for group

Table 1. Effect of *in ovo* feeding maltose with different concentration on hatchability (%) and body weight (g) of broilers

	Group				
	1	2	3	4	5
Hatchability (%)	90.00	89.16	88.33	60.83	0
19E	41.02 ^b	42.01 ^{ab}	43.23 ^a	41.15 ^b	43.76 ^a
	±0.88	±1.15	±1.16	±1.17	±1.29
20E	45.08 ^b	47.55 ^a	48.48 ^a	46.79 ^{ab}	48.39 ^a
	±0.60	±0.70	±0.70	±0.66	±0.68
Hatch	42.75 ^b	45.04 ^a	45.50 ^a	44.01 ^b	-
	±0.55	±0.97	±0.63	±0.77	

^{a-b} means within the same row with no common superscript are significantly different ($P < 0.05$).

5 exhibited greater villi length, width and villus surface area compared with the control group, and the group 4 was the most prominent (Table 2, Fig. 1C).

Nutrition *in-ovo* can provide embryos with nutrients being not adequate for optimal growth and development of embryos and for development after hatching (Uni and Ferket 2005, Foye *et al.* 2006). The oral consumption of carbohydrate can supply the energy and improve the growth of embryos (Uni *et al.* 2005, Foye *et al.* 2006, Tangara *et al.* 2010, Wei *et al.* 2010). In our study, *in-ovo* feeding a given maltose solution to embryos, hatchling weights increased by 2.9 to 6.4% over controls (Table 1). Uni and Ferket (2004) found IOF solutions containing carbohydrates and protein increased hatchling weights by 3% to 7%. Providing carbohydrates and β -hydroxy- β -methyl butyrate to late-term embryos increased hatching weights by 5 to 6% (Uni *et al.* 2005). Tako *et al.* (2004) observed that in IOF the carbohydrates to the late-term embryo led to a 3.2% increase of body weight (BW) at day of hatch compared with the control. Hatchling weight is a major predictor of marketing weight in chickens (Uni *et al.* 2005). Wilson (1991) reported that each 1 g of increase in BW at hatch leads to 8 to 13 g of increase in BW at marketing. But excessively high maltose concentration cannot improve the growth of embryos, reversely, reduce hatchability (maltose, 300 g/L), and even result in embryos death (maltose, 400 g/L) (Table 1), which may be resulted from the higher osmotic pressure of *in-ovo* feeding maltose solution.

Previous studies suggested that final growth in the bird is directly proportional to an early high specific growth rate of supply organs (Lijia 1981). As the intestine is the primary supply organ and must function very early in life to ensure survival and the rapid growth and development of hatchlings (Uni *et al.* 2003). *In ovo* feeding nutrients to the embryo can promote the development of the small intestine. This study examined the effect of IOF of different concentration maltose on day 17.5 of incubation on small intestinal development of chickens during pre- and post-hatch periods. The

Table 2. Effect of *in-ovo* feeding maltose with different concentration on jejuna villus length, width and surface area of broiler from day19 of incubation until hatch

	Age	Group				
		1	2	3	4	5
Villus length (μm)	19E	117.60 ^c ±46.48	132.30 ^b ±21.42	139.20 ^{ab} ±16.56	144.00 ^a ±11.21	120.00 ^c ±11.80
	20E	155.00 ^b ±78.12	167.33 ^b ±22.81	183.33 ^a ±32.76	143.67 ^c ±18.56	139.67 ^c ±20.04
	Hatch	313.00 ^d ±64.59	378.21 ^a ±58.97	365.17 ^b ±38.88	348.67 ^c ±26.49	-
Villus width (μm)	19E	28.33 ^b ±10.80	30.87 ^b ±4.42	35.41 ^a ±3.20	28.67 ^b ±7.67	29.74 ^b ±6.94
	20E	33.20 ^b ±14.36	32.33 ^b ±8.84	42.49 ^a ±6.32	30.67 ^b ±3.52	46.03 ^a ±2.58
	Hatch	35.33 ^c ±7.48	39.67 ^b ±4.42	47.60 ^a ±6.46	36.67 ^b ±6.78	-
Villus surface area (μm^2)	19E	10461 ^c ±1100	12824 ^b ±825	15477 ^a ±1527	12963 ^b ±1278	11206 ^{bc} ±2272
	20E	16158 ^c ±1600	16987 ^c ±1380	24460 ^a ±909	13836 ^d ±1509	20187 ^b ±1432
	Hatch	34723 ^d ±1673	47111 ^b ±1510	54580 ^a ±1048	40147 ^c ±912	-

^{a-d} means within the same row with no common superscript are significantly different ($P < 0.05$).

morphological measurements of jejuna villi revealed that IOF maltose can enhance intestinal development by increasing the size of villi (Fig. 1). On the day of hatch villus height, width and surface area of all IOF treatments exhibited increasing compared with the control group, and which were significant increased for the injection 200 g/L maltose group by 17, 35 and 57%, respectively ($P < 0.05$) (Table 2). Uni *et*

al. (2004) observed that jejunal villi height was 50% greater, 48 h after IOF carbohydrate than controls. Tako *et al.* (2004) found that carbohydrate IO fed embryos had 50% elevation in villi surface area prior to hatch relative to control. Smirnov *et al.* (2006) observed that IOF carbohydrate increased villus surface area at hatch by about 27%. This rapid intestinal growth may be due to accelerated processes of enterocyte

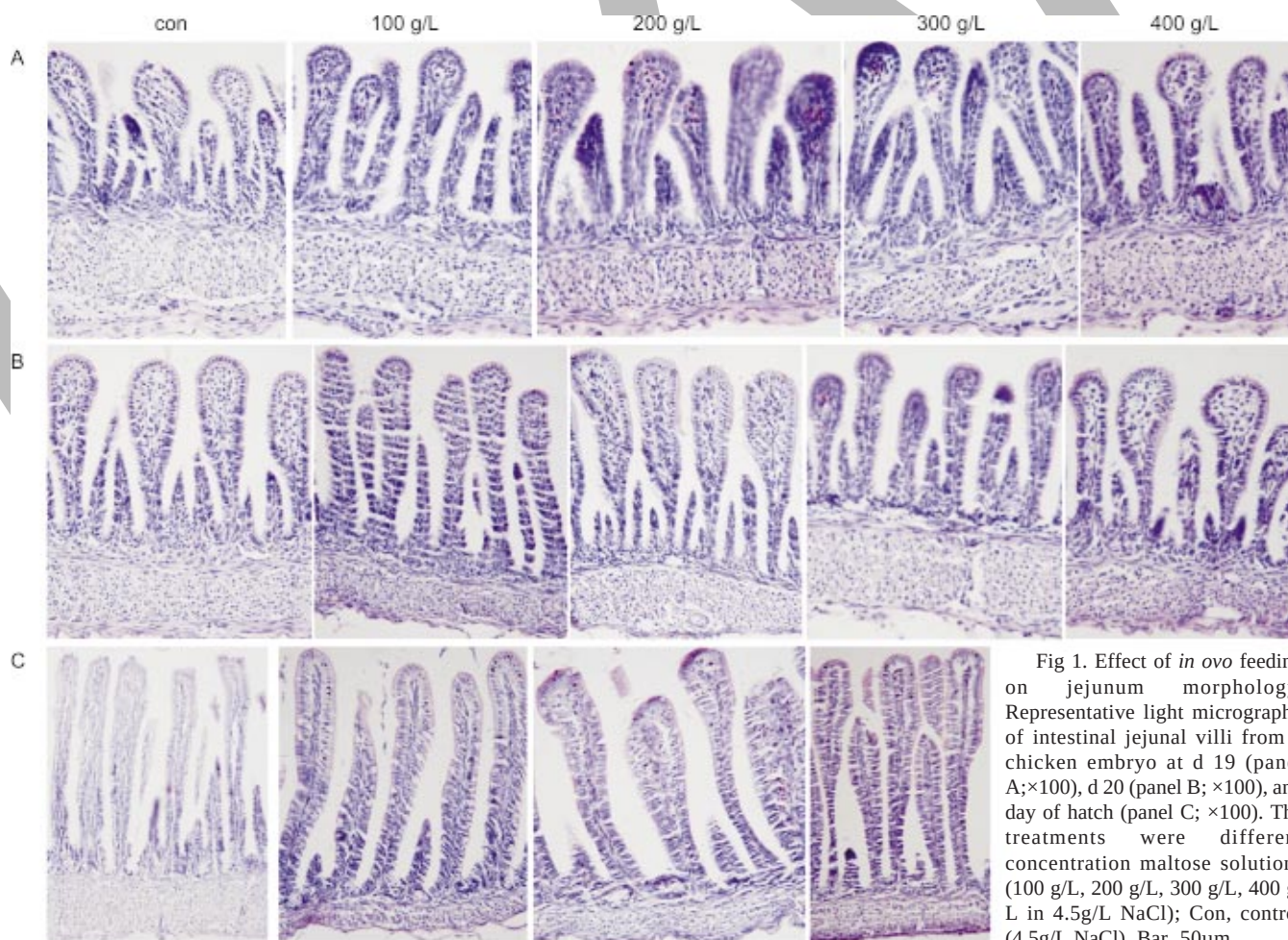


Fig 1. Effect of *in ovo* feeding on jejenum morphology. Representative light micrography of intestinal jejunal villi from a chicken embryo at d 19 (panel A; $\times 100$), d 20 (panel B; $\times 100$), and day of hatch (panel C; $\times 100$). The treatments were different concentration maltose solutions (100 g/L, 200 g/L, 300 g/L, 400 g/L in 4.5g/L NaCl); Con, control (4.5g/L NaCl). Bar, 50 μm .

proliferation and differentiation (Geyra *et al.* 2001), increased brush boarder enzyme activities, and glucose cross-membrane transport activities (Uni and Ferket 2004), or upregulated the expression of sodium-glucose transporter (SGLT1), SGLT5 and SGLT6 (Li *et al.* 2008, Foye *et al.* 2009). In addition, Smirnov *et al.* (2006) found the expansion of surface area that occurs with villus growth probably relative to the higher level expression of mucin mRNA. Therefore, this early feeding will accelerate gut development and ultimately functional maturity. The rapid functional development of the gut will be beneficial to increase nutrient digestion and assimilation that will result in body weight elevation.

ACKNOWLEDGMENTS

This work was supported by the special funds of Central Colleges basic scientific research operating expenses and the State-Level Animal Science Experiment Demonstration Center Foundation.

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